IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

SMITH et al.

Serial No. **09/662,462**

Filed: **September 15, 2000**

Atty. Ref.: **2551-49**

Group: **1655**

Examiner: **Goldberg**

For: **NUCLEIC ACID PROBES AND METHODS FOR DETECTING CLINICALLY IMPORTANT FUNGAL PATHOGENS**

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August 10, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

AMENDMENT

Responsive to the Official Action dated April 10, 2001, entry and consideration of the following amendments and remarks are requested; the period for response having been extended up to and including August 10, 2001, by submission of the requisite petition and fee, attached.

IN THE SPECIFICATION

Amend the specification as follows:

Page 15, delete the paragraphs spanning lines 9-22 and insert the following therefor:

-- ITS5 (forward): GGAAGTAAAAGTCGTAACAAGG (SEQ ID NO:44) and

ITS4 (reverse): TCCTCCGCTTATTGATATGC (SEQ ID NO:45),

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ITS5 (forward): GGAAGTAAAAGTCGTAACAAGG (SEQ ID NO:44) and

ITS2 (reverse): GCTGCGTTCTTCATCGATGC, (SEQ ID NO:46),

ITS1 (forward): TCCGTAGGTGAACCTGCGG (SEQ ID NO:47) and

ITS4 (reverse): TCCTCCGCTTATTGATATGC (SEQ ID NO:45),

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ITS1 (forward: TCCGTAGGTGAACCTGCGG (SEQ ID NO:47) and

ITS2 (reverse): GCTGCGTTCTTCATCGATGC (SEQ ID NO:48),

ITS3 (forward): GCATCGATGAAGAACGCAGC (SEQ ID NO:49) and

ITS4 (reverse): TCCTCCGCTTATTGATATGC (SEQ ID NO:45).--

Insert the attached Sequence Listing in place of the copy of the Sequence Listing filed September 15, 2000.

IN THE CLAIMS

Amend the claims as follows.

Cancel claims 1-11 and 19-23, without prejudice.

Add the following claims:

--24. (new) Method to detect and identify fungal pathogenic species in a sample, comprising at least the following steps:

(i) releasing, isolating and/or concentrating the nucleic acids of the fungal pathogens possibly present in the sample,

(ii) if necessary, amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least one of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2)

TATCAACTTGTCACACCAGA (SEQ ID NO:3)

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

(iv) detecting the hybridization complexes formed in step (iii), and

(v) identifying the fungal pathogenic species present in said sample, based on the hybridization complex formed.

25. (new) Method according to claim 24, wherein the ITS region in step (ii) is limited to the ITS-1 region, and wherein the probes in step (iii) are chosen from the following set of probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2),

TATCAACTTGTCACACCAGA (SEQ ID NO:3),

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary nucleic acids of said probes.

26. (new) Method according to claim 24, wherein said fungal universal primer pair is chosen from the following group of primer pairs:

ITS5: GGAAGTAAAAGTCGTAACAAGG (SEQ ID NO:44) and

ITS4: TCCTCCGCTTATTGATATGC (SEQ ID NO:45),

ITS5: GGAAGTAAAAGTCGTAACAAGG (SEQ ID NO:44) and

ITS2: GCTGCGTTCTTCATCGATGC (SEQ ID NO:46),

ITS1: TCCGTAGGTGAACCTGCGG (SEQ ID NO:47) and

ITS4: TCCTCCGCTTATTGATATGC (SEQ ID NO:45),

ITS1: TCCGTAGGTGAACCTGCGG (SEQ ID NO:47) and

ITS2: GCTGCGTTCTTCATCGATGC (SEQ ID NO:48),

ITS3: GCATCGATGAAGAACGCAGC (SEQ ID NO:49) and

ITS4: TCCTCCGCTTATTGATATGC (SEQ ID NO:45).

27. (new) Method according to any of claims 24 to 26, wherein said fungal pathogen is a *Candida* species, and wherein the probes of step (iii) are chosen from

among SEQ ID NO 1, 2 and 3 for *C. albicans*, SEQ ID NO 4 and 5 for *C. parapsilosis*, SEQ ID NO 6 for *C. tropicalis*, SEQ ID NOs:7 and 8 for *C. kefyr*, SEQ ID NO 9 for *C. krusei*, SEQ ID NO:10 for *C. glabrata*, and SEQ ID NOs:11, 12 and 13 for *C. dubliniensis*.

28. (new) Method according to claim 27 to detect *Candida albicans* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to at least one probe chosen from among SEQ ID NOs:2, 3, 33, 34 and 35,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. albicans* is present in said sample, based on the formation of said hybridization complex.

29. (new) Method according to claim 27 to detect *Candida parapsilosis* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to at least one probe chosen from among SEQ ID NOs:4 and 5,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. parapsilosis is present in said sample, based on the formation of said hybridization complex.*

30. (new) Method according to claim 27 to detect *Candida tropicalis* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to at least one probe chosen from among SEQ ID NOs:6 and 36,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. tropicalis* is present in said sample, based on the formation of said hybridization complex.

31. (new) Method according to claim 27 to detect *Candida kefyr* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to at least one probe chosen from among SEQ ID NOs:7 and 8,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. kefyr* is present in said sample, based on the formation of said hybridization complex.

32. (new) Method according to claim 27 to detect *Candida krusei* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to at least one probe chosen from among SEQ ID NOs:9 and 37,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. krusei* is present in said sample, based on the formation of said hybridization complex.

33. (new) Method according to claim 27 to detect *Candida glabrata* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to a probe represented by SEQ ID NO:10,

(ii) detecting the hybridization complexes formed,

(iii) inferring that *C. glabrata* is present in said sample, based on the formation of said hybridization complex.

34. (new) Method according to claim 27 to detect *Candida dubliniensis* in a sample, said method comprising

(i) hybridizing the nucleic acids present in the sample to at least one probe chosen from among SEQ ID NOs:11, 12, 13 and 38,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. dubliniensis* is present in said sample, based on the formation of said hybridization complex.

35. (new) Method according to clam 24 wherein the probes of step (iii) are immobilized to a solid support.

36. (new) Method according to claim 24 for the simultaneous detection and differentiation of at least two fungal pathogenic species in one single hybridization step, including

(i) releasing, isolating and/or concentrating the nucleic acids of the fungal pathogens possibly present in the sample,

(ii) amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least two of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2),

TATCAACTTGTCACACCAGA (SEQ ID NO:3),

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

wherein said probes have been immobilized to a solid support on specific locations,

(iv) detecting the hybridization complexes formed in step (iii),

(v) identifying the species present in the sample by the location of the hybridization signal on the solid support.

37. (new) Isolated oligonucleotide molecule having a nucleotide sequence represented by any of SEQ ID NOs:2 to 13 or 33 to 38.

38. (new) Isolated oligonucleotide molecule according to claim 37, for use as a species specific primer or probe in the detection of one of the following fungal pathogens: *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida kefyr, Candida krusei, Candida glabrata, Candida dubliniensis, Aspergillus flavus, Aspergillus versicolor, Aspergillus nidulans, Aspergillus fumigatus, Cryptococcus neoformans and Pneumocystis carinii.*

39. (new) Method according to claim 24, wherein the sample is a blood sample, and wherein step (i) comprises

incubation of the blood sample with lysis buffer (10 mM Tris-HC1, pH 7.5, 10 mM EDTA, 50mM NaCl), followed by centrifugation and removal of the supernatant, and vortexing of the resuspended cell pellet in the presence of glass beads.

40. (New) Method according to claim 24, wherein the oligonucleotide probes comprise a homopolymer tail which is added at the 3' or 5' extremity of the probe.--

REMARKS

Reconsideration is requested.

Claims 1-11 and 19-23 have been canceled, without prejudice. New claims 24-40 have been added. Claims 12-18 and 24-40 are pending. No new matter has been added.

The specification has been amended to include the sequence identifiers for the sequences originally disclosed on page 15. Moreover, the sequences disclosed on page 15 have been added to the Sequence Listing and a revised copy of the same is attached. The attached paper and computer-readable copies of the Sequence Listing are the same. No new matter has been added. A separate Letter to this effect is attached.

Claim 3 has been rewritten as new claim 26 to include sequence identifiers in response to the Examiner's comments spanning pages 4-5 of the Office Action dated April 10, 2001 (Paper No. 10). The application is submitted to comply with the sequence rules.

The Section 112, first paragraph, rejection of claims 1-2 and 19-23 is moot in view of the above. Moreover, the Section 112, second paragraph, rejection of claims 1-11 and 19-23 is moot in view of the above. The claims are submitted to be supported by enabling disclosure. Moreover, the claims are submitted to be definite. The claims have been amended with the Examiner's comments in mind. Basis for the amendments to the claims may be found throughout the specification. Basis may be found, for example, on page 20, lines 21-22 and 27-28; page 21, lines 1-2, 7-8, 13-14, 19-20 and 25-26; and page 14, lines 25-28. No new matter has been added.

The claims have been amended to advance prosecution without prejudice.

The Section 103 rejections of claims over Williams et al (1996), Botelho et al (1994), and Hogan (1997); and Lott et al (1995) and Lott (1997) in view of Hogan (1997)

are moot. The claims are submitted to be patentable over the cited art and consideration of the following in this regard is requested.

The applicants submit that Williams and Botelho teach the alignment of *C. albicans, C. tropicalis* and *C. krusei* ITS sequences. Lott teaches the ITS2 sequence of *C. albicans, C. parapsilosis* and *C. tropicalis*. Lott teaches the ITS2 sequence of *C. dubliniensis*. Hogan teaches how probes can be designed based on the identification of variable regions within aligned sequences. The Examiner believes that it would have been obvious to design the probes of the invention, based on the *Candida* sequences and their alignment and based on the teaching by Hogan.

The applicants respectfully disagree and urge consideration of the following in this regard.

The applicants believe that the designing of probes that are able to differentiate the 7 *Candida* species was not at all straightforward. Initially, not all *Candida* ITS sequences were known at the time of the present invention. Moreover, many other ITS sequences from other fungal species, possibly also present in a patient's sample, were not known. Therefore, it was not at all certain that a probe theoretically designed by the method of Hogan and based on the aligned sequences of Williams, Botelho or Lott, would have been able to differentiate the *Candida* species from each other and from other fungal species. In fact, the inventors experienced a great amount of cross-reactivity of the probes with unknown ITS sequence from other *Candida* species and from other fungal species present in the patient's samples. The inventors have designed and tested various probes in order to obtain the set of probes of the present

invention that does not cross-hybridize with other fungal species. The probes recited in claim 24 are thus selected because of their specificity for only *Candida* species, while not cross-hybridizing with other fungal species. Hogan does not teach how to obtain a probe that does not cross-hybridize with other fungal species of which the sequence is not known.

Furthermore, the applicants note that the probes of the present invention are designed to specifically function in the hybridization assay of the present invention.

The assay of the present invention uses probes that are immobilized, for example, to a solid support by means of a homopolymer tailing sequence which is added (enzymatically or chemically) at the 3' or 5' extremity of the probe. Depending on the tailing of the probe, a different probe sequence has to be used in the assay of the present invention. Attached is a table that shows a comparative experiment with various probes tailed at their 3' or 5' end. This table clearly illustrates that not all probe sequences, theoretically designed, function in the hybridization assay of the invention. The probes of the present invention have been designed to function specifically as a 3' or a 5' tailed probe in the assay of the present invention. Hogan does not teach how to design probes that function in this specific hybridization assay. The probes of the presently claimed invention have been selected to function specifically in the assay of the present invention and would not have been obvious in view of the cited art.

Thus, although *Candida* ITS sequences may have been known and a method for probe design, based on variable regions, may have been known, it was not at all

obvious to design the probes of the present invention that function in the hybridization assay of the invention and that do not cross-hybridize with other fungal species.

The claims are submitted to be patentable over the cited art.

The Section 103 rejections of claims 19-22 over Williams et al (1996), Botelho et al (1994), Lott et al (1995), Lott (1997), Hogan (1997), Fujita et al (1995) and Jordan (2000) are moot in view of the above. The claims are submitted to be patentable over the cited art and consideration of the following in this regard is requested.

Initially the applicants note the Examiner has combined six references in alleging the claimed invention was obvious. That the Examiner required so many references in attempting to establish a *prima facie* case of obviousness appears to support the applicants' belief that it would not have been obvious to make the presently claimed invention.

As for the teachings of the cited art, the applicants note that Fujita teaches a microtiterplate hybridization assay with dioxigenin- and biotin labeled probes. Jordan teaches a solid support detection of PCR amplified DNA. According to the Examiner, these disclosures would have motivated the ordinarily skilled person to detect fungal pathogens using probes from variable regions of the *Candida* species on a solid support.

The applicants submit however that, as indicated above, probes were selected to specifically hybridize in the hybridization assay of the invention while not showing any cross-hybridization with other fungal species. Although Fujita and Jordan teach a hybridization assay on a solid support, they do not teach how to select probes that

function as 3' or 5' tailed probes in the hybridization assay of the invention. Neither Fujita nor Jordan teach how to select probes that do not cross-hybridize with unknown fungal sequences. The probes of the present claims are thus selected to function specifically in the assay of the present invention while not showing any cross-hybridization with other fungal species. Thus, although *Candida* ITS sequences may have been known and the principles of the immobilization of probes may have been known, it was not at all obvious to design the specific probes of the present invention that function in the hybridization assay of the invention and that do not cross-hybridize with other fungal species. The claims are submitted to be patentable over the cited art.

The Section 103 rejection of claim 23 over Williams et al (1996), Botelho et al (1994), Lott et al (1995), Lott (1997), Hogan (1997), Fujita et al (1995) and Tomblin et al (1986) is moot. The claims are submitted to be patentable over the cited art and consideration of the following in this regard is requested.

Fujita and Tomblin teach the isolation of fungal pathogens from blood. According to the Examiner, this teaching would have motivated the ordinarily skilled person to perform the method of claim 23. In this respect the applicants note that, as indicated above, the probes of the present invention were selected to specifically hybridize in the hybridization assay of the invention while not showing any cross-hybridization. Neither Fujita nor Tomblin teach how to select probes that function as 3' or 5' tailed probes in the hybridization assay of the invention. They also do not teach how to select probes that do not cross-hybridize with unknown fungal sequences. The probes in claim 23 are thus selected to function specifically in the assay of the present invention and do not

show any cross-hybridization with other fungal species. Thus although *Candida* ITS sequences may have been known and the isolation of fungal pathogens from blood may have been known, it was not at all obvious to design the specific probes of the present invention that function in the hybridization assay of the invention and that do not cross-hybridize with other fungal species. The claims are submitted to be patentable over the cited art.

Return of a further initialed copy of the PTO-1449 Form considered April 2, 2001, with the Examiner's initials in the left-hand column indicating specific consideration of the following: WO 98 11257, WO 99 06596, WO 96 21741, WO 98 50584, and WO 95 29260, is requested. Specifically, while the PTO-1449 Form returned with Paper No. 10 includes the Examiner's signature and date considered as well as the Examiner's initials in the left-hand column next to most of the references, the Examiner has not initialed the specifically listed WO documents such that it may be unclear as to whether the Examiner has considered these documents. The Examiner is requested to return a further initialed copy of the PTO-1449 Form, pursuant to MPEP §609.

In view of the above, the claims are submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

B. J. Sadoff
Reg. No. **36,663**

BJS:eaw
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100